Supplemental Figures for Escobar, Desai et al.,

Fig. S1: S641D fails to enhance phosphorylation by CK1 in a αE-cat C-terminal fragment. **A)** Schematic of full-length (FL) GST-αE-cat and a C-terminal truncation (C-term, amino acids 450-906) GST-αE-cat, as well as S641A and S641D phospho-mutant and -mimic mutations on αE-cat. **B)** Autoradiograph of [γ-^{32}P]-ATP in vitro kinase labeling of recombinant FL and S641D C-term αE-cat. Time course is shown in minutes (’). Coomassie-stained gel bands of purified GST-tagged αE-cat proteins shown below. **C)** Quantification of (B), as described in Fig. 2. Columns represent three independent experiments over more than one protein preparation. Numeric value of the relative fold-increase shown. **D)** Autoradiograph of [γ-^{32}P]-ATP in vitro kinase labeling of recombinant FL and S641A C-term αE-cat. **E)** Quantification of (D) as described in Fig. 2. Columns represent three independent experiments over more than one protein preparation. Numeric value of relative fold-increase shown.

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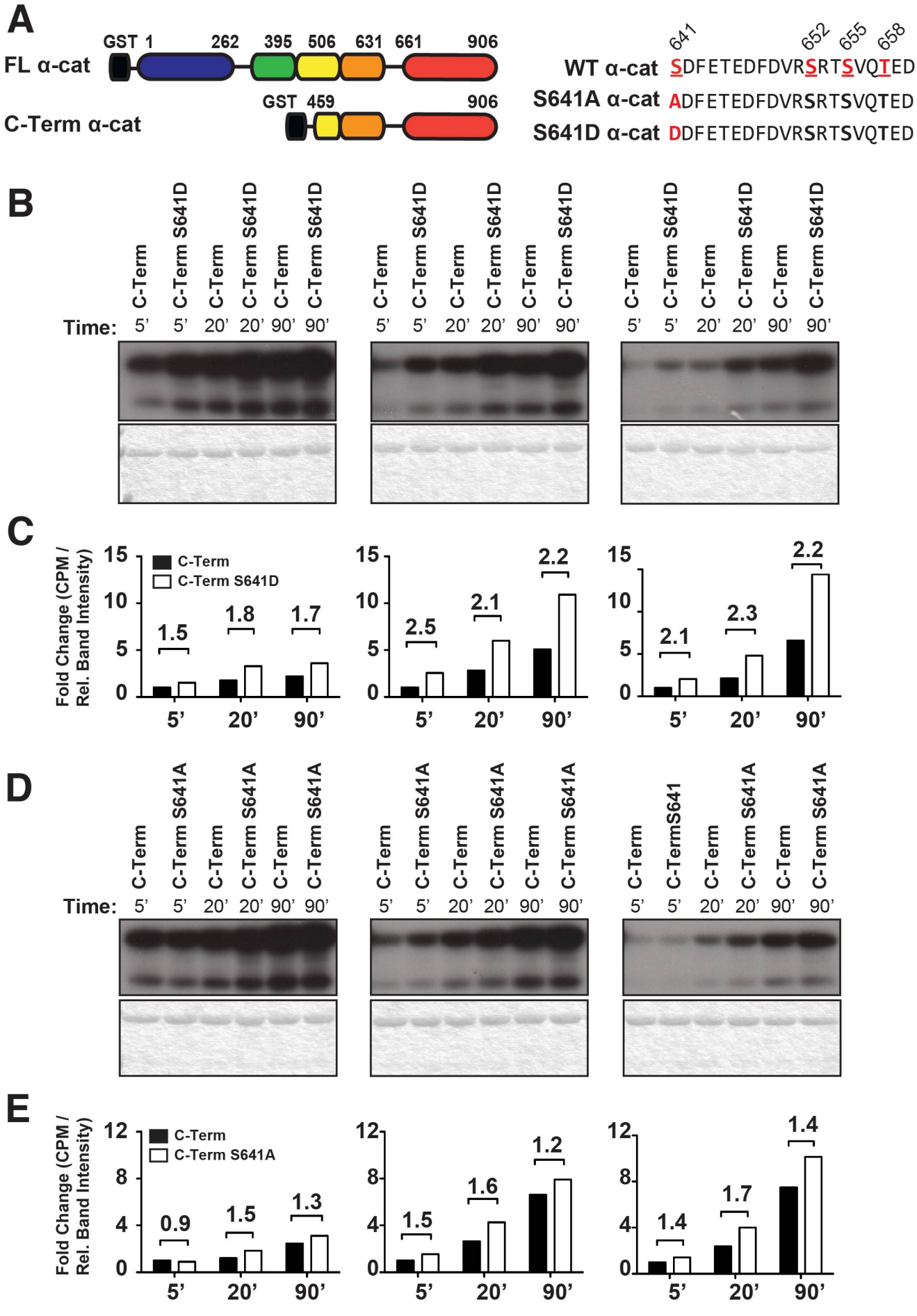

Fig. S2: Phospho-domain charge status does not impact cell aggregation or cadherin/catenin complex assembly. A) Western blot showing MDCK knock down (KD)/reconstitution cell clones. These are independent clonal lines of those shown in Fig. 3. **B)** Image of hanging drop aggregation assay. Aggregates are quantified by the number of particles greater than threshold (5 cells per aggregation particle (**C**)) and distribution of aggregate particles arranged by percent of particles displaying <4, 5-100 or >100 cells per particle (**D**). Error bars represent standard error of the mean of three technical replicates. Similar results were observed for independent replicates. **F)** Neutral protease (Dispase) Mechanical Disruption Assay, in the presence of a range of low (0.1 mM) to normal (1.0 mM) calcium. Y-axis reflects the number of macroscopic epithelial fragments counted after shaking (Methods). Error bars represent standard error of the mean from three independent experiments performed on per cell line. **G)** Western blot of MDCK knock down (KD)/reconstitution cell clones immunoprecipitated with antibodies to GFP and probed for β-catenin and E-cadherin. Statistical significance was assessed by Student's t-test, where $p<0.05$ (*) or $p<0.001$ (***).

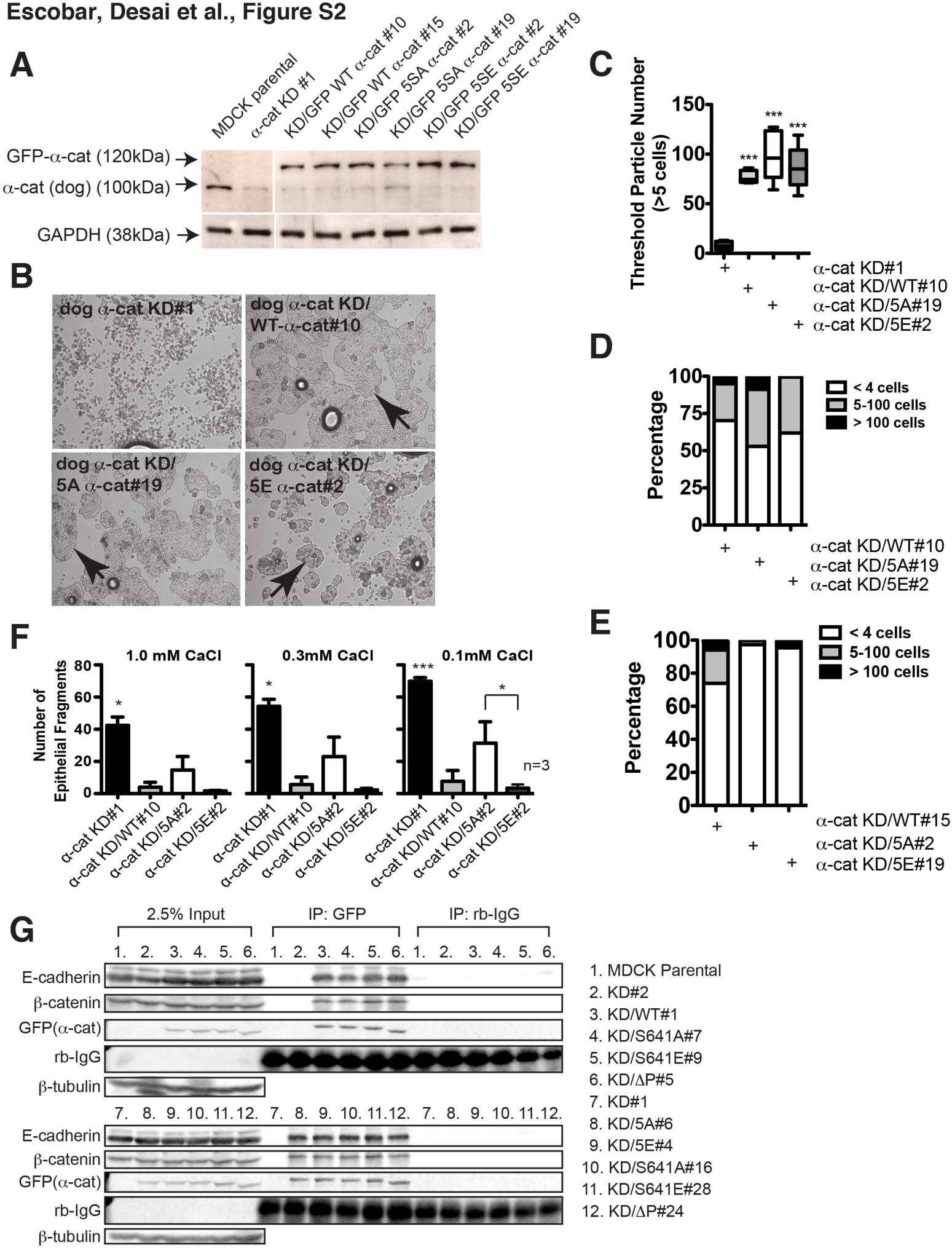
A
MDCK parental
α-cat KD #1
KD/GFP WT α-cat #10
KD/GFP WT α-cat #15
KD/GFP 5SA α-cat #2
KD/GFP 5SA α-cat #19
KD/GFP 5SE α-cat #2
KD/GFP 5SE α-cat #19
GFP-α-cat (120kDa)
α-cat (dog) (100kDa)
GAPDH (38kDa)
B
dog α-cat KD#1
dog α-cat KD/ WT-α-cat#10
dog α-cat KD/ 5A α-cat#19
dog α-cat KD/ 5E α-cat#2
C
Threshold Particle Number (>5 cells)
150
100
50
0
***
***
***
α-cat KD#1
α-cat KD/WT#10
α-cat KD/5A#19
α-cat KD/5E#2
D
Percentage
100
75
50
25
0
< 4 cells
5-100 cells
> 100 cells
α-cat KD/WT#10
α-cat KD/5A#19
α-cat KD/5E#2
E
Percentage
100
80
60
40
20
0
< 4 cells
5-100 cells
> 100 cells
α-cat KD/WT#15
α-cat KD/5A#2
α-cat KD/5E#19
F
1.0 mM CaCl
0.3mM CaCl
0.1mM CaCl
Number of Epithelial Fragments
80
60
40
20
0
*
*
***
*
n=3
α-cat KD#1
α-cat KD/WT#10
α-cat KD/5A#2
α-cat KD/5E#2
G
2.5% Input
IP: GFP
IP: rb-IgG
1. 2. 3. 4. 5. 6.
E-cadherin
β-catenin
GFP(α-cat)
rb-IgG
β-tubulin
7. 8. 9. 10. 11. 12.
1. MDCK Parental
2. KD#2
3. KD/WT#1
4. KD/S641A#7
5. KD/S641E#9
6. KD/ΔP#5
7. KD#1
8. KD/5A#6
9. KD/5E#4
10. KD/S641A#16
11. KD/S641E#28
12. KD/ΔP#24

Fig. S3: Expression of Drosophila αCat P-linker mutants in the follicular epithelium and border cells. Ovaries of adult survivors from whole animal rescue experiments (see Figure 5A) were stained for HA to detect transgenic constructs and Armadillo (Arm). Left panels show face on and side views of the follicular epithelium whereas right panels show border cell clusters during migration. Distribution of P-linker mutants isoform of αCat is similar to that of αCat::HA or Arm.

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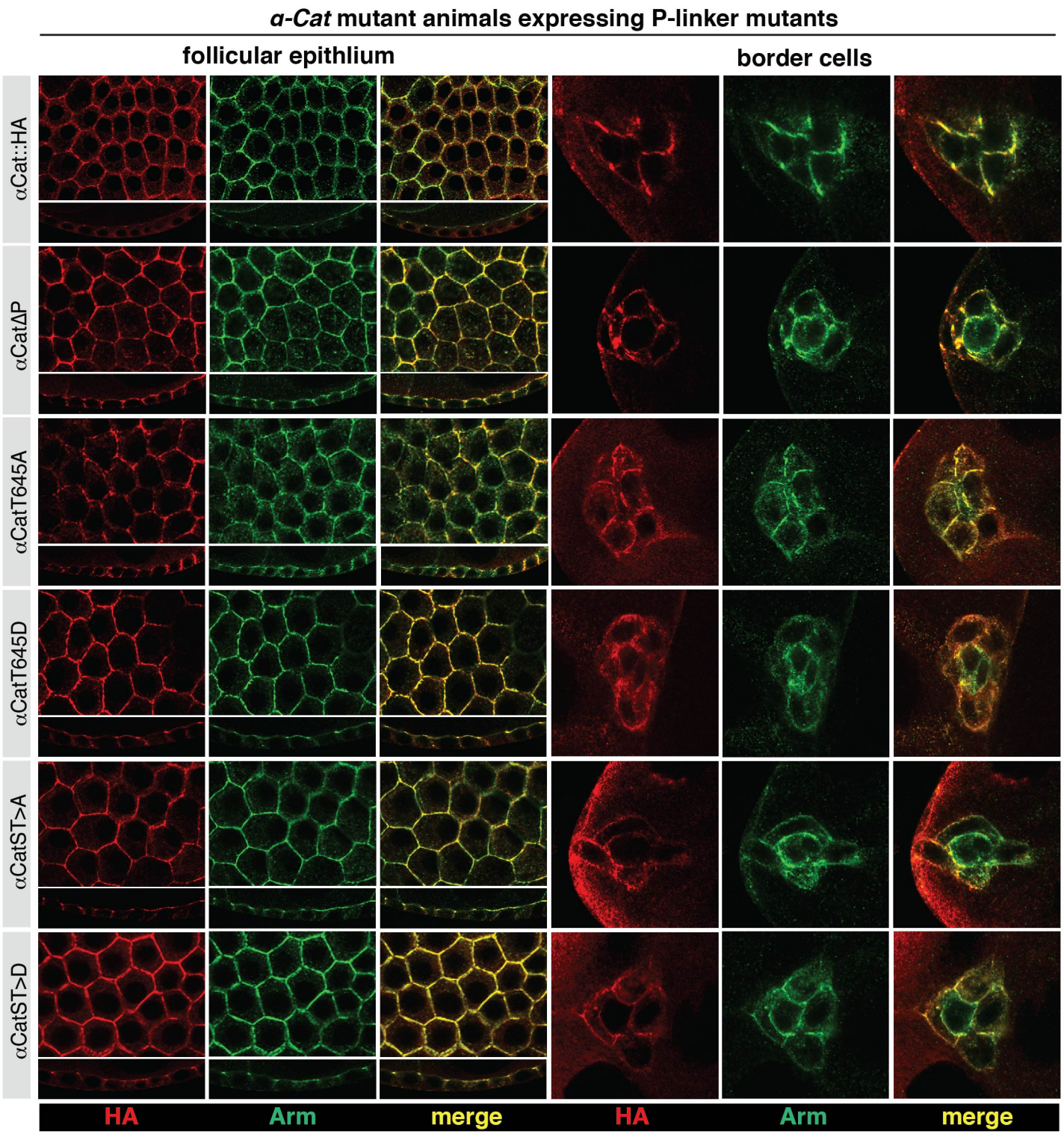


Fig. S4: Representative movie time points from MDCK sheet migrations.

Montage representing selected movie time-points (00:00, 01:40, 03:20, and 05:00 hours) during MDCK sheet migration analysis. One representative cell line for each GFP::αE-cat construct is shown. Colored tracks represent paths of individual cells in the wound front. Scale bar, 100 μm.

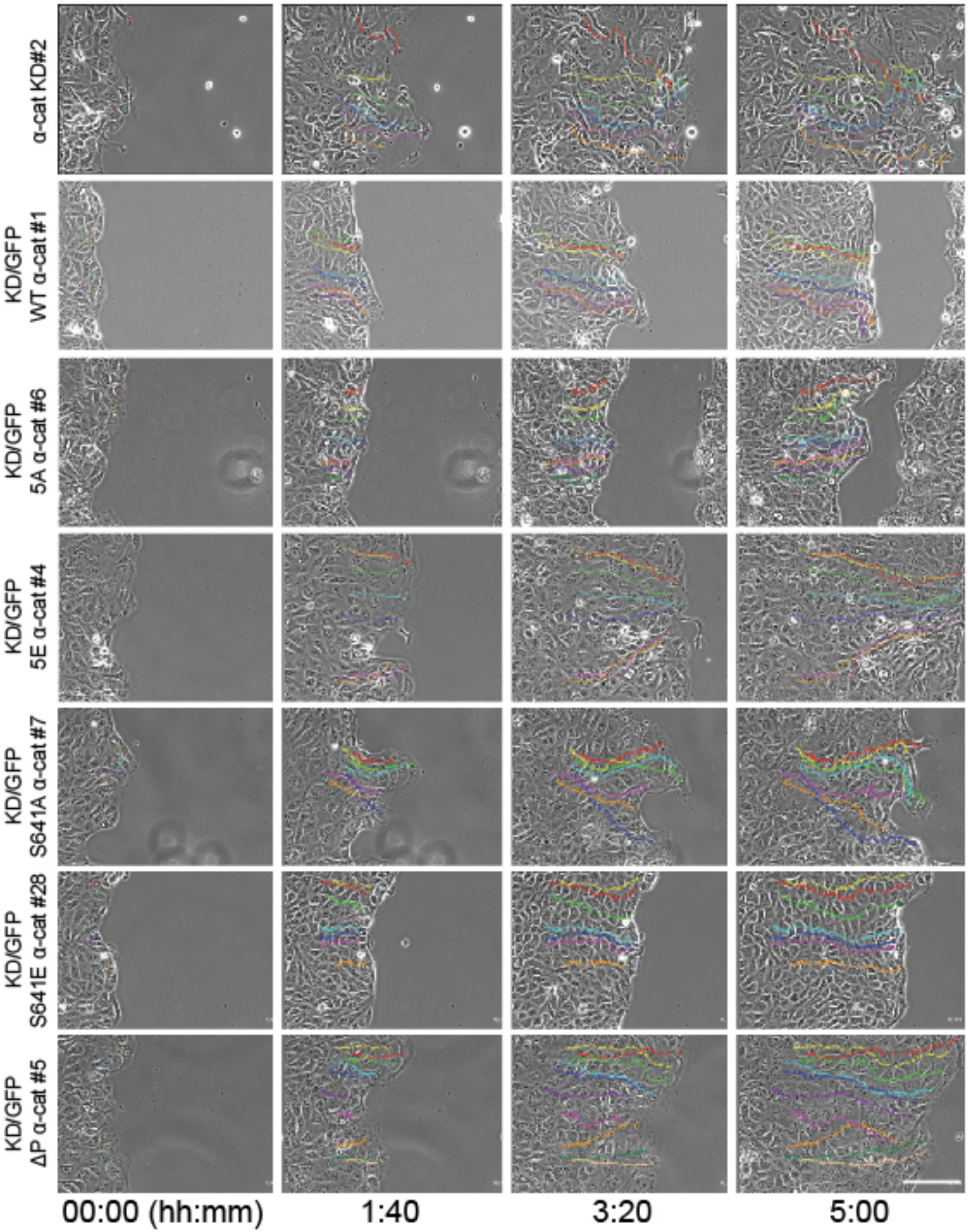
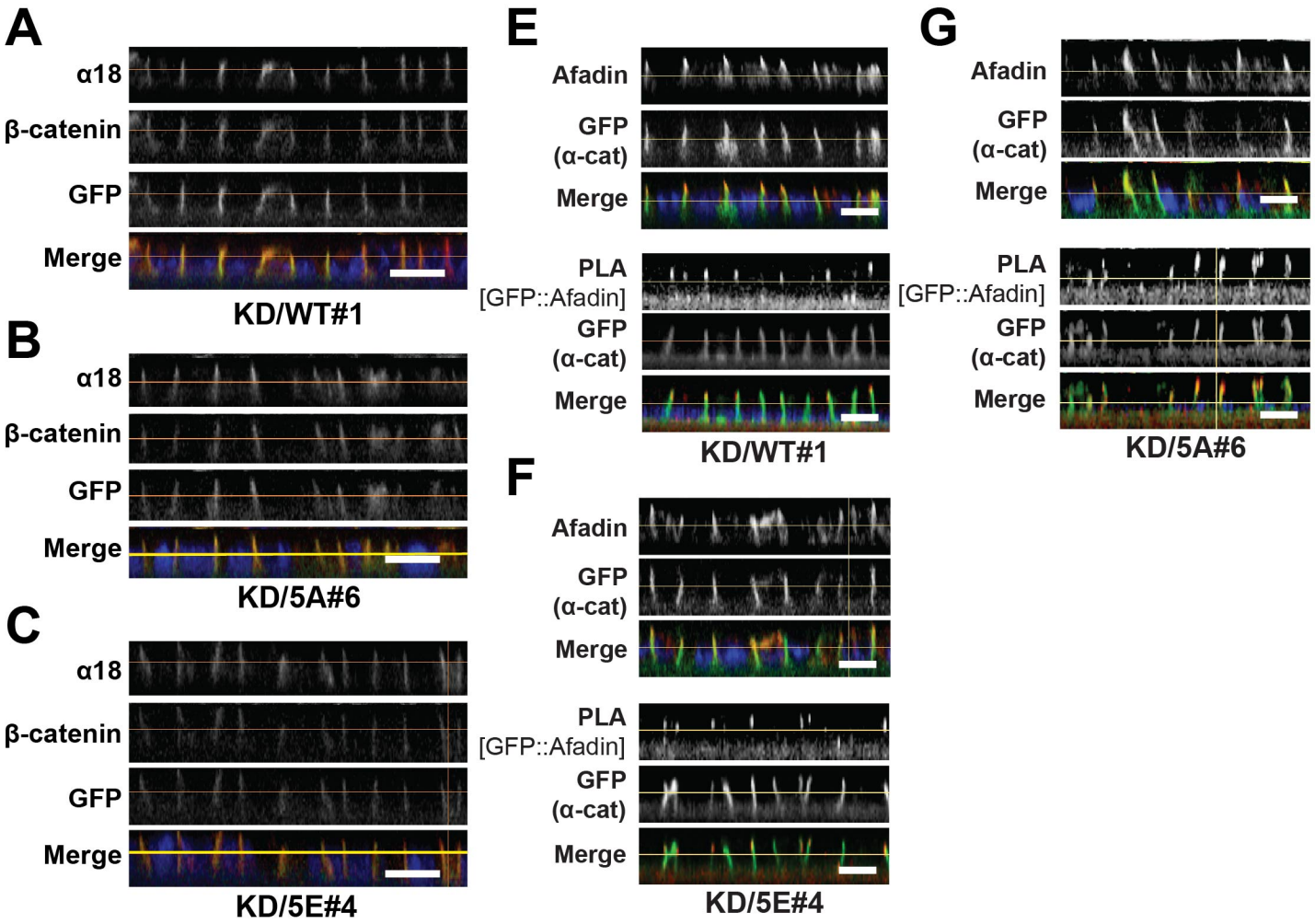

α-cat KD#2
KD/GFP
WT α-cat #1
KD/GFP
5A α-cat #6
KD/GFP
5E α-cat #4
KD/GFP
S641A α-cat #7
KD/GFP
S641E α-cat #28
KD/GFP
ΔP α-cat #5
00:00 (hh:mm)
1:40
3:20
5:00

Fig. S5: Mutation within the P-linker of αE-cat does not affect steady-state localization of afadin or recognition by conformation-dependent αE-cat antibody (α18), and trypsinolysis of purified αE-cat proteins. A-C) Localization of α18 conformation-dependent antibody in MDCK cells expressing wild-type (KD/WT#1), phospho-mutation (KD/5A#6) or phospho-mimic αE-cat (KD/5E#4), shown in z-stack confocal sections. **D)** Quantification of (A-C) in terms of α18 intensity (arbitrary units, A.U.) per cell. **E-G)** Cellular localization of GFP-αE-cat and afadin (upper panels) and localization of Proximity Ligation Assay (PLA) signal between αE-cat and afadin (lower panels) along sites of cell-cell contact is shown in z-stack confocal sections in MDCK cells expressing wildtype (KD/WT#1), phospho-mutant (KD/5A#6) or phospho-mimic (KD/5E#4) αE-cat. **H)** Quantification of afadin intensity in (E-G), in terms of A.U. per cell. **I)** Quantification of PLA signal intensity in (E-G), in terms of A.U. per cell. No difference in PLA signal intensity due to phospho-mutation or phospho-mimic αE-cat was observed. Fluorescence intensity quantifications based on at least 3 or more fields of view. **J)** Limited proteolysis of GST-affinity purified, thrombin-cleaved WT-, 5A- or 5D- αE-cat proteins. Coomassie-stained SDS-PAGE of proteins incubated for time indicated with 0.01 mg/mL trypsin. This gel represents the experiment that was subjected to N-terminal sequencing, as in (Fig. 7E).


A
α18
β-catenin
GFP
Merge
KD/WT#1
B
α18
β-catenin
GFP
Merge
KD/5A#6
C
α18
β-catenin
GFP
Merge
KD/5E#4
E
Afadin
GFP (α-cat)
Merge
PLA [GFP::Afadin]
GFP (α-cat)
Merge
KD/WT#1
F
Afadin
GFP (α-cat)
Merge
PLA [GFP::Afadin]
GFP (α-cat)
Merge
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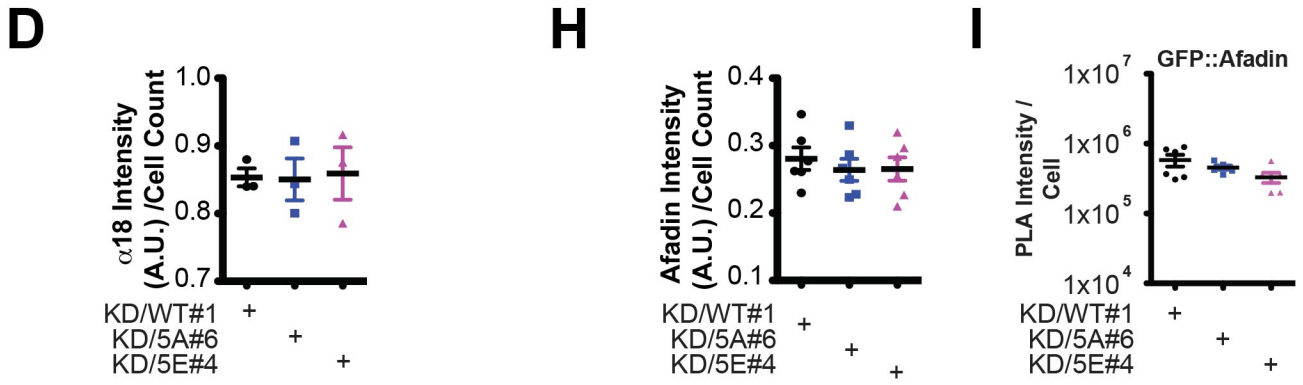
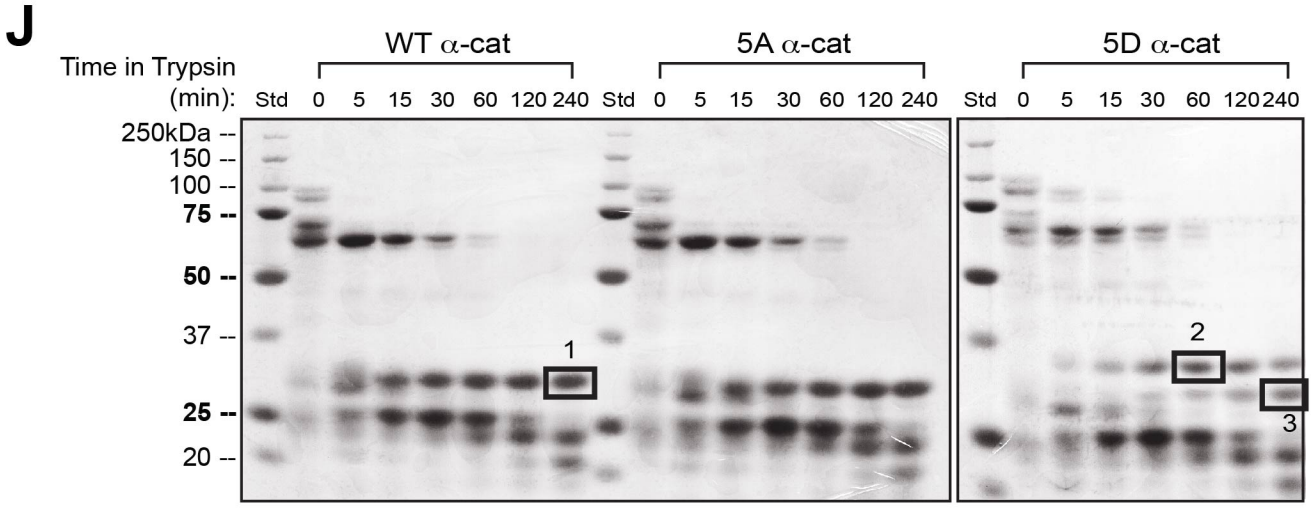
G
Afadin
GFP (α-cat)
Merge
PLA [GFP::Afadin]
GFP (α-cat)
Merge
KD/5A#6


D
α18 Intensity (A.U.) /Cell Count
1.0
0.9
0.8
0.7
KD/WT#1 +
KD/5A#6 +
KD/5E#4 +
H
Afadin Intensity (A.U.) /Cell Count
0.4
0.3
0.2
0.1
KD/WT#1 +
KD/5A#6 +
KD/5E#4 +
I
GFP::Afadin
PLA Intensity / Cell
1x10^7
1x10^6
1x10^5
1x10^4
KD/WT#1 +
KD/5A#6 +
KD/5E#4 +


J
Time in Trypsin (min):
WT α-cat
5A α-cat
5D α-cat
Std 0 5 15 30 60 120 240
250kDa
150
100
75
50
37
25
20
1
2
3